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Isoprene cancer risk and the time pattern of dose administration

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Abstract

Most statistical risk assessment models assume that equal doses, measured on a scale such as mg/kg/day, create equal tumor risks. This equivalent dose metric (EDM) hypothesis allows risks to be extrapolated from high concentrations to low concentrations and from one species, sex, and strain to another, since it implies that all administered dose histories corresponding to the same total dose create the same risk. This paper tests the EDM hypothesis using data on tumor rates in $B6C3F_1$ mice administered isoprene via inhalation. Its major conclusion is that the EDM hypothesis does not hold for isoprene. For example, it appears that exposure concentration has a greater impact on tumor rates than weeks of exposure. To predict tumor probabilities, the time pattern of dose administration must be considered. The asymmetric effects of concentration, hours-per-day, and number of days of exposure on tumor risks imply that complex dynamic risk models may be needed to accurately describe dose-time-response relations. The traditional concept of a dose-response relation as a static curve relating a numerical summary of dose to a numerical summary of response probability is probably not predictively useful for chemicals such as isoprene, and extrapolations of risk based on the EDM hypothesis could be misleading for such chemicals.

Keywords: Isoprene; Risk assessment; Carcinogenesis; Dose metrics; Dose-response modeling

1. Introduction

This paper uses data on isoprene-induced carcinogenesis to test a key assumption of applied cancer risk assessment: the existence of "equivalent dose metrics" (EDMs) that permit valid extrapolations of cancer risks from one set of exposure conditions to another within and between species. This hypothesis is fundamental for much of regulatory risk assessment (Kolloru, 1995). For example, the U.S. Department of Health and Human Services (USDHHS, 1986) summarized the situation as follows:

> The empirical approach to species extrapolation in carcinogenesis has tended to rely on the use of a milligram per kilogram (mg/kg) body weight per day (that is, based on body size), parts per million (ppm) in the diet (based on concentration), milligrams per square meter of surface area per day (that is, based on body surface area), or mg/kg in a lifetime (based on size and species longevity) dosage scale. Low-dose risk estimates can vary by as much as 40-fold, depending on the choice of a particular dosage scale. No single scale has been consistently employed or agreed upon by scientists in the field.

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Despite uncertainties about which dose metric (i.e. "dosage scale") is most appropriate for specific applications, the concept that *some* dose metric exists on which equal doses produce equal risks has been widely accepted and applied.

Several aspects of the EDM hypothesis are tested in this paper using data from a recently completed stop exposure study on isoprene (Placke et al., 1995). The main finding is that no EDM exists that permits valid extrapolation (or interpolation) of isoprene risks across different time patterns of exposure even within a single species and strain. Two implications are that, (i) dynamic risk models, capable of accounting for differences in biological responses to different time patterns of exposures, are needed to draw valid inferences about isoprene-induced risks; and (ii) the processes that mediate between isoprene inhalation and onset of isoprene-induced cancer are probably nonlinear. Other implications are discussed at the end of the paper.

1.1. The EDM hypothesis and its implications

Since risk assessors commonly use EDMs to extrapolate from tumor rates observed under experimental study conditions to the exposure conditions relevant to human situations (USDHHS, 1986; Kolloru, 1995), it is important to define the EDM in a way that leads to accurate predictions. Definitions widely used today represent dose as a product of powers of quantities such as administered concentration (ppm), exposure duration (hours per day and number of days) and animal body weight raised to a power — typically, -1, $-2/3$, or $-3/4$ power, depending on which of various allometric scaling methods is followed. Indeed, the "product law" of dimensional analysis (Lin and Segel, 1988; Doucet and Sloep, 1992) implies that cumulative hazard for tumor induction should be proportional to a product of powers of the factors that determine risk. To assess the validity of the EDM hypothesis as clearly as possible, it is convenient to focus on responses in a single species, strain, and sex exposed via a single route, thus eliminating these factors as possible sources of variation. The following testable predictions — or hypotheses to be tested — can then be made:

(H1) Tumor incidence rates should be higher at higher exposure concentrations.
(H2) Tumor incidence rates should increase with longer exposure durations.
(H3) If cumulative dose provides an adequate basis for predicting risk, then increasing hours-per-day of exposure and proportionally reducing the number of weeks of exposure should leave tumor incidence rates unchanged.
(H4) If cumulative dose provides an adequate basis for predicting risk, then increasing the concentration and proportionally reducing the number of weeks (or the hours-per-day) of exposure should leave tumor incidence rates unchanged.
(H5) If risk is a smooth, linear-at-low-doses function of cumulative dose (as in the multistage model and some other well-known statistical dose-response models), then doubling the exposure concentration or doubling the exposure duration should approximately double risk when doses are low enough so that isoprene pharmacokinetics and metabolism are approximately linear and tumor incidence rates are small (e.g. <0.01). More precisely, doubling either factor should approximately double cumulative tumor hazard, H, which is related to tumor probability, P, by the probability identity $P = 1 - \exp(-H)$.
(H6) If risk (hazard rate or cumulative hazard) is expressed by a product of powers of exposure factors (such as concentration, hours per day, and number of weeks of exposure), then tumor incidence rates should be proportional to a power of exposure concentration when other factors (specifically, hours per day and number of weeks of exposure) are held constant.

Each of these properties is implied by the EDM hypothesis for some common choices of the dosage scale defining the units in which dose is measured. For example, if dose is measured either in mg/kg-day or in total mg/lifetime of administered dose (and if tumor risk at each anatomic site is uniquely determined by dose, as is assumed in drawing a dose-response function), then all of these properties would hold. These predictions will be tested using responses of male $B6C3F_1$ mice exposed to isoprene via inhalation.

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Table 1
Summary of experimental design and tumor incidence data in male B6C3F1 mice

Group	(ppm)	(weeks)	(h/day)	HEPAD	BRONAD	OTHAD	HEPCAR	BRONCAR	HISTSARC	HEMSARC
1	0	0	8	0.22	0.22	0.14	0.18	0	0	0.04
2	10	80	8	0.24	0.32	0.12	0.12	0.02	0.04	0.10
3	70	40	8	0.29	0.16	**0.30***	0.22	0	0.04	0.14
4	70	80	8	0.30	0.08	0.18	0.18	0.04	0.04	0.08
5	140	40	8	**0.44***	0.20	**0.28***	0.20	0.02	0.02	0.14
6	280	20	8	0.36	0.32	**0.36***	0.24	0.06	**0.16***	0.12
7	280	80	8	**0.48***	0.26	**0.34***	0.32	0.02	0.08	0.08
8	700	80	8	**0.54***	**0.46***	**0.54***	0.34	**0.14***	0.04	**0.20***
9	2200	20	4	**0.44***	0.28	**0.38***	0.24	0.04	**0.10***	**0.16***
10	2200	80	4	**0.42***	0.30	**0.56***	0.30	0.06	**0.14***	0.10
11	2200	40	8	**0.57***	**0.59***	**0.65***	**0.37***	0.06	**0.14***	0.08
12	2200	80	8	**0.60***	**0.60***	**0.72***	0.32	**0.14***	0.04	0.14

Columns 2–4 summarize the exposure factors defining each of the dose groups 1–12. The remaining columns show the fraction of animals in each dose group (row) that were found to have each tumor type (column heading) at necropsy.
*Indicates tumor incidence rates are significantly greater than in the control group ($P \leq 0.05$ by Fisher's exact test).
HEPAD, hepatocellular adenomas; BRONAD, alveolar-bronchiolar adenomas; OTHAD, other (non-liver, non-lung) adenomas (most of which were adenomas of the Harderian gland); HEPCAR and BRONCAR, liver and lung carcinomas, respectively; HISTSARC, histiocytic sarcoma (aggregated across anatomic sites for purposes of this summary table); HEMSARC, hemangiosarcoma (aggregated across anatomic sites).

2. Materials and methods

2.1. Experimental design and materials

The statistical design of the experiment used to test the EDM hypothesis is summarized in the first four columns of Table 1. The first column identifies the experimental dose group, each of which initially had 50 male $B6C3F_1$ mice assigned to it. The second, third, and fourth columns give the exposure concentration, total number of weeks of exposure, and hours per day of exposure for each dose group, respectively. Animals were exposed for 5 days per week for 4 or 8 h per day. Mice were necropsied after spontaneous death or after scheduled sacrifice. The inhalation chambers used to expose mice to carefully regulated concentrations of isoprene in accord with the statistical design, and other details of the experimental materials and methods, are given in Placke et al. (1995). The main idea of the statistical design was to create several sets of dose groups (e.g. 4, 5, and 6; 10 and 11; and 7 and 9, to a close approximation) that achieved the same total administered dose through very different combinations of the exposure scenario factors (i.e. concentration, hours per day, and weeks of exposure). Within the budget constraints for the experiment, this design allows for several independent tests (conducted in different dose groups) of the key implications of the EDM hypothesis.

2.2. Data analysis methods

The statistical analyses performed are described more fully elsewhere (Cox, 1994). The main analyses and statistical methods were as follows.

2.2.1. Comparisons of quantal response rates

The mean response rate (i.e. the tumor incidence rate) for each type of tumor in each dose

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group was compared to the corresponding rate in the control group using Fisher's exact test for nonparametric comparison of count data in 2×2 tables (Siegel, 1956). (Nearly identical results were also achieved using t-tests for comparisons of proportions. However, nonparametric tests were used wherever possible to avoid making potentially controversial modeling assumptions.) In each exposure group, these comparisons tested the null hypothesis that the tumor incidence rate was not elevated (for each tumor type) against the alternative hypothesis that it was elevated. Similar comparisons were made for other pairs of dose groups (e.g. to compare response rates in dose group 4 against response rates in dose group 6). More general hypotheses, stating that aggregate tumor rates (combined across tumor types and/or dose groups) were elevated in dose groups 7–12 compared to groups 1–6 were tested using the sign test (Siegel, 1956). For example, sign tests established that rare tumors, such as forestomach tumors, are significantly elevated in the higher dose groups, even though there are not enough such tumors to establish a significant elevation in any individual dose group compared to the control group.

2.2.2. Survival data analysis

All-cause and cause-specific Kaplan-Meier survival curves were compared in pairs using a variety of non-parametric tests (Peto and Peto, Gehan's Wilcoxon, F-test, and Cox's test) (Andersen et al., 1993). These tests took into account time-to-tumor information and censoring due to competing risks — potentially important phenomena not captured in the quantal comparisons.

2.2.3. Two-way interactions among risk factors and responses

Associations between potential risk factors (concentration, h/day, weeks of exposure, scheduled sacrifice vs. unscheduled death, and sex of mouse) and potential response variables (time of death, presence of various tumors at death, body weight at death) were explored using several measures of ordinal association (Kendall's Tau, Spearman's rank correlation coefficient, and the gamma coefficient). Rigorous tests of association were then performed using sign tests to detect significant associations between risk factors and responses within dose groups. The sign of the ordinal association between each risk factor and each response variable was quantified in each of the 12 dose groups. Then, the number of dose groups in which the association had the same sign was used to reject or not reject the null hypothesis of no association in favor of the alternative hypothesis of a non-random association between the risk factor and the response variable. As an example, time of death (which may be considered a possible risk factor as well as a response variable) and presence of hepatocellular adenoma at death were found to be positively associated (mean age at death was greater in male mice that died with hepatocellular adenomas than in male mice that died without hepatocellular adenomas) in all 12 dose groups. The probability that such an extreme outcome would occur by chance in the absence of a true association is only $P = 2/4096 = 1/2048$. The null hypothesis of no association is rejected at any significance level $\geqslant 1/2048$. Since the association holds *within* each dose group, it cannot be explained away by suggesting that both the risk factor and the response variable are associated with one or more other factors (such as concentration) that vary only *between* dose groups.

2.2.4. Two-way interactions among response variables

Pairwise interactions among response variables within dose groups were detected and tested using the same methodology (measures of ordinal association followed by hypothesis testing using a sign test) as just described for pairwise interactions between risk factors and response variables. For example, these tests established whether the presence of one type of tumor makes it significantly more or less likely that a different type of tumor will also be present.

2.2.5. Multivariate analyses

Multiway interactions among risk factors (hours per day, number of weeks, and exposure

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Table 2
Frequency counts of forestomach, heart, and lymphoma tumors in male B6C3F1 mice

Group	(ppm)	(weeks)	(h/day)	Forestomach papillomas	Forestomach squamous cell carcinomas	Heart hemangio sarcomas	Malignant lymphocytic lymphomas	Total mixed cell lymphomas	Lymphoid leukemias	Row total
1	0	0	8	0	1	0	1	1	0	3
2	10	80	8	0	0	0	1	0	1	2
3	70	40	8	0	0	0	2	1	0	3
4	70	80	8	0	0	0	3	1	0	4
5	140	40	8	0	0	0	0	1	1	2
6	280	20	8	0	0	0	4	4	0	8
7	280	80	8	0	1	1	4	1	0	7
8	700	80	8	1	0	4	3	1	0	9
9	2200	20	4	0	1	2	2	2	0	7
10	2200	80	4	1	1	1	2	2	0	7
11	2200	40	8	2	0	1	4	1	0	8
12	2200	80	8	1	3	2	2	2	0	10

concentration) and among responses (tumor incidence rates at different sites) were explored visually using k-means clustering and a variety of interaction graphs. In addition, log-linear models were used to detect multiway interactions and to confirm pairwise interactions discovered using the sign test. A novel machine-learning method drawn from the intersection of artificial intelligence and computational statistics (Biggs et al., 1991) was also used to learn classification trees and tumor prediction rules. These trees and rules provide a nonparametric summary of multiway interactions among risk factors and response variables. The need for such analysis was shown by the fact that up to five variables could interact significantly in determining the incidence rates of certain tumors. Such high-order interactions could not be detected using conventional techniques such as log-linear analysis, since there were not enough observations.

The relatively sophisticated methods and tests (e.g. survival data analysis and classification trees) generally confirmed and extended the results of simpler comparisons. The simplest methods (sign tests and quantal comparisons) suffice to make most of the main points, except those involving high-order interactions. For simplicity and brevity, this paper will focus on the quantal response data summarized in Tables 1 and 2.

3. Results

3.1. Tumor quantal response data

In B6C3F1 mice, chemically induced tumors most often occur in the liver, lung, and forestomach, in that order (Huff, 1991). Columns 5, 6, 8, and 9 of Table 1 present tumor incidence rate data for benign and malignant tumors of liver and lung, respectively. For example, the 0.44 shown for dose group 5 in the "HEPAD" column indicates that 44% of the mice in dose group 5 (22 out of 50 animals) were found with hepatocellular adenomas at necropsy. Columns 5 and 6 of Table 2 present frequency count data for tumors in the forestomach. Table 2 also presents data on heart hemangiosarcomas and lymphomas.

These tumors are especially interesting for isoprene because they occur at elevated rates in B6C3F1 mice exposed to the structurally similar chemical 1,3-butadiene (Melnick et al., 1990). Moreover, heart hemangiosarcomas have a very

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low spontaneous incidence rate. Finally, Table 1 also summarizes data from several other tumors that occurred at significantly elevated rates—specifically, histiocytic sarcomas and hemangiosarcomas, pooled across various anatomic sites for the sake of brevity; and adenomas at sites other than the liver and lung. (Most of these other adenomas occurred in the Harderian gland. Histiocytic sarcomas were significantly elevated at higher doses for the kidney, lung liver, lymph nodes, marrow, and spleen.)

The main results of the statistical data analyses were as follows:

(1) Sufficiently high administered concentrations of isoprene are positively associated with increased tumor risk, both in the aggregate (i.e. pooling tumors across anatomic sites) and at specific sites, including the lung, liver, forestomach, heart, and Harderian gland among male mice. The lowest observed effect level (LOEL) occurred at either 70 ppm (unless the elevated response in Harderian gland tumors in dose group 3 was due to chance) or 140 ppm. Thus, 10 ppm was a no observed effect level (NOEL) in this study. (This is true for both sexes. This paper focuses on males, since females were exposed only to dose groups 1, 2, and 4.) The location of the LOEL is ambiguous due to the statistical problem of "multiple comparisons": the apparently "significant" elevation of Harderian gland adenomas in dose group 3 (captured in the "OTHAD" column of Table 1) might have occurred by chance, given the large number of comparisons being made and the fact that no significant increase was detected in dose group 4, which had a strictly higher dose. The fact that higher concentrations are significantly positively associated with increased tumor incidence rates at several sites was established using tests for significantly positive ordinal association (e.g. Spearman's rank correlation coefficient) between concentration and tumor incidence rates in dose groups exposed for 8 h per day for 80 weeks. As shown in Table 1, Fisher's exact test (as well as *t*-tests for proportions) confirm this finding. Thus, hypothesis H1—that concentration is positively associated with tumor risk—is supported by the data.

(2) Longer durations of exposure are not necessarily associated with significantly increased tumor risks. For example, comparison of the quantal responses for dose groups (rows) 3 and 4 in Table 1 suggests that doubling the weeks of exposure from 40 to 80 does not significantly increase (and may even decrease) tumor incidence rates when the exposure concentration is 70 ppm. When dose groups 6 and 7 are compared, it is seen that quadrupling the weeks of exposure from 20 to 80 does not significantly increase all tumor incidence rates, which in fact are smaller in dose group 7 than in dose group 6 for 5 of the 7 tabulated response categories. Even when more weeks of exposure are associated with higher tumor incidence rates, as in dose groups 9–12, the increases in tumor hazard are generally far less than proportional to the increases in weeks of exposure between the dose groups being compared (e.g. 9 and 10, or 11 and 12). Survival data analysis confirms the lack of statistically significant differences between Kaplan-Meier survival curves when weeks of exposure are doubled or quadrupled, both at the highest concentration level (2200 ppm) and at 70 ppm (dose groups 3 and 4), for both all-cause and tumor-specific survival curves. However, at 280 ppm, the all-cause and the lung and liver adenoma survival curves for dose group 7 lie significantly to the left of the corresponding curves for dose group 6. Thus, the effect on lung adenoma, liver adenoma, and all-cause death rates of increasing weeks of exposure appears to be minimal at low (70 ppm) and high (2200 ppm) concentrations, but significant at intermediate (280 ppm) concentrations. This pattern is inconsistent with predictions H2 and H5 and with any product-of-powers model in which tumor hazard is expressed as a product of powers of other factors multiplied by some power of weeks of exposure. The experimental design did not permit the effects of animal age at the start and end of dosing to be distinguished from the effects of exposure duration, leaving this as a possible direction for further experimental investigation.

(3) Decreasing weeks of exposure (e.g. from 80 to 20) and proportionally increasing administered isoprene concentration (e.g. from 70 ppm to 280

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ppm) increases tumor risk, rather than leaving it unchanged. For example, Table 1 shows that all tumor incidence rates are higher in group 6 than in group 4, even though both dose groups received the same cumulative dose. Survival data analysis confirms that the cause-specific survival curves for deaths with lung adenomas and with adenomas present (treating all other deaths as censoring events) are significantly leftward-shifted in groups 5 and 6 compared to group 4. These comparisons refute hypothesis H4 and demonstrate that cumulative dose does not provide an adequate basis for predicting isoprene-induced tumor risks.

(4) At 2200 ppm, reducing weeks of exposure from 80 to 40 and proportionally increasing hours per day of exposure from 4 to 8 increases tumor risk, rather than leaving it unchanged. Comparison of the quantal response rates in dose groups 10 and 11 in Table 1 suggest that dose group 11 has a higher incidence of tumors. Survival data analysis confirms this, and shows that deaths with lung adenomas, in particular, pose a higher risk (leftward-shifted cause-specific survival curve) in group 11 compared to group 10. This refutes hypothesis H3, and provides a second way in which cumulative dose fails to provide an adequate basis for predicting isoprene-induced tumor risks.

(5) The increase in tumor hazard when concentration is increased from 10 ppm to 70 ppm (for exposures of 8 h per day, 5 days per week, for 80 weeks) is nonsignificant. By contrast, the increase in tumor hazard when concentration is increased from 280 to 2200 (also for 8 h/day and 80 weeks) — slightly less than an 8-fold increase — is highly statistically significant. Indeed, even a 4-fold increase from 70 ppm to 280 ppm, creates a very significant increase in hazard rates. The increase in hazard rates when concentration increases from 70 ppm to 2200 ppm is much less when the duration is 40 weeks than when it is 80 weeks (compare groups 3 and 11 and 4 and 12). Taken together, these observations suggest that tumor hazard is not simply the product of several other factors with some power of exposure concentration, in contradiction to hypothesis H6.

The following additional results, although not specifically related to testing the EDM hypothesis, are also worth noting as part of understanding the profile of tumor risks created by inhalation of isoprene.

(6) There is no evidence of a lymphoma response analogous to the one for 1,3-butadiene. On the other hand, heart hemangiosarcomas have an elevated incidence rate for isoprene concentrations in excess of 280 ppm (See Table 2).

(7) Tumors at different anatomic sites do not occur statistically independently of each other. In particular, hepatocellular adenomas are positively associated with lung (alveolar-bronchiolar) adenomas and with other adenomas (e.g. of the Harderian gland) within dose groups, but are negatively associated with histiocytic sarcomas and hemangiosarcomas (pooled across anatomic sites) and with lung carcinomas. Similarly, lung adenomas are negatively associated with lung carcinomas (i.e. presence of one makes presence of the other less likely than it otherwise would be).

(8) The ratio of lung tumor incidence to liver tumor incidence is significantly different in different dose groups, showing that the probabilities of tumors at different anatomic sites depend on the dose factors in different ways.

4. Discussion

4.1. Discussion of findings

Several of the findings just described were unexpected. For example, in 10 out of the 12 dose groups — all except groups 4 and 5 — the incidence of liver adenomas is higher among animals with lung adenomas than among animals without them. This may seem surprising, since tumors at different sites are often negatively associated due to mutual censoring. Both lung and liver adenomas are positively associated with age at death, however, and survival data analysis indicates that neither increases death rate. Thus, censoring of one by the other does not occur. A possible interpretation of the positive association between liver adenomas and adenomas at other

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sites is that some animals produce relatively high amounts of adenoma-inducing metabolites via hepatic metabolism. Another unexpected finding was that, in both liver and lung, adenomas are negatively associated with carcinomas within dose groups, perhaps because adenomas progress to carcinomas.

Various strong two-way and multiway interactions were also found using the machine-learning program (Biggs, 1991). Among other things, this program discovered that a randomly selected mouse with a lung adenoma is more than four times as likely to belong to dose group 6 as to belong to dose group 4 (and about twice as likely to belong to dose group 11 as to dose group 10). A mouse with a histiocytic sarcoma is also about four times more likely to belong to dose group 6 than to belong to dose group 4. Asked to discover rules for discriminating between mice in dose groups 4 and 6, the program found that, in dose group 6, 20% of the mice had both lung adenomas and liver adenomas, while in dose group 4, this combination did not occur at all, reflecting the weak association between liver tumors and isoprene exposure below 700 ppm. (In dose group 5, it occurred among 6% of the mice.) Similarly, a mouse with a lung adenoma and a Harderian gland adenoma is more than six times as likely to belong to dose group 11 as to dose group 10. These findings confirm that the response profiles in these different, arithmetically "equivalent" dose groups are sufficiently different to allow useful probabilistic classification of which dose group an animal belongs to, given its profile of tumors. A striking finding was that significant high-order interactions occurred among many of the variables. For example, to predict the probability of a lymphoma, it is useful to know the animal's sex (females have a much higher spontaneous incidence rate of lymphomas), whether histiocytic sarcomas were present (if so, lymphoma is unlikely), the concentration (if the animal is male, in which case concentrations over 140 ppm are associated with increased risk of lymphoma, although the risk is not significantly different among dose groups 6–12), and, in females, whether liver adenomas and carcinomas were present (each increases the likelihood of lymphoma), whether the animal lived until scheduled sacrifice, and the weight at death (which tends to be greater than 35 g if and only if lymphoma is present). Such complex interactions among factors are well summarized in classification trees. Simple statistical models (e.g. logistic regression models or other models that combine factors through weighted summation) cannot adequately describe such complex, nonlinear interactions.

4.2. Implications for isoprene risk modeling

The data presented in this paper have potential implications for the probable mechanisms of isoprene-induced cancer, for risk assessment modeling, and for risk assessments that use the EDM hypothesis. Each topic is briefly discussed next.

The fact that extending the number of weeks of exposure by a factor of 2 or 4 does not greatly increase lifetime probability of a tumor (for dose groups 3 and 4 and for dose groups 9 and 10) appears to be incompatible with a classic genotoxic initiator model of carcinogenesis. In the classic genotoxic model, an administered carcinogen causes random mutations in stem cells to occur throughout the exposure period, leading to a production of initiated cells that should be approximately proportional to the duration (number of weeks) of exposure (Kopp-Schneider and Portier, 1994). By contrast, a pattern in which most of the animals that will respond will do so even after a relatively brief number of weeks (e.g. 20 weeks) of exposure, as seems to be the case for male B6C3F1 mice exposed to isoprene, is consistent with the following possible explanations. Either,

(i) The mice have very heterogeneous metabolism, pharmacokinetics, and/or pharmacodynamic responses (such as DNA repair processes) for exogenously administered isoprene. (This is consistent with the observation that mice with liver adenomas are at increased risk for lung adenomas, since these mice may be the ones that most effectively metabolize isoprene to reactive carcinogenic forms or that least effec-

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tively detoxify reactive metabolites such as diepoxides.)
(ii) Isoprene exposure may act primarily on cells that have already been made susceptible (e.g. "initiated") by other sources. In this case, once the susceptible cells have been promoted beyond a critical stage, continued exposure to isoprene will have relatively little additional effect.

Both of these possible explanations are speculative. Whatever the correct mechanism(s) of isoprene carcinogenicity may be, however, it seems unlikely that a purely genotoxic mechanism would give the observed time-dose-response pattern. If such a mechanism prevails at low doses, its quantitative properties will probably be different from those observed at high doses, where other mechanisms may be active. All of the observations of increased tumor risks in this study involved mice exposed for at least 20 weeks. Whether there is a minimum number of weeks of exposure that is required to elicit a tumor response remains to be determined.

The isoprene data have several potentially important implications for the practice of risk assessment. First, the widespread practice of extrapolating risks from a relatively high-concentration, brief-duration exposure in animals to much lower-concentration, longer-duration exposure settings in humans appears to be inappropriate. Ignoring issues of interspecies extrapolation, this procedure does not work reliably even within a single species and strain, as shown by the fact that dose group 6 is more hazardous (and produces a significantly different response profile) than dose group 4. Secondly, the role of hours per day of exposure — or, more generally, the pattern of exposures within a day — may have a larger proportional effect on risk than number of weeks of exposure. For isoprene, the disproportionately greater effect of 8 h per day compared to 4 h per day may be attributed to depletion of cofactors such as GSH necessary for detoxification, or to differences in pharmacodynamics after more hours of exposure. This issue should ideally be examined for a wider range of doses than was possible in this experiment.

Finally, the quantitative prediction that risk should increase roughly in proportion to total carcinogenic metabolites produced does not appear to be plausible for isoprene. This is illustrated by the fact that dose groups 4 and 6, which probably produce similar amounts of metabolites [since metabolism is approximately linear over this range of concentrations (Peter et al., 1990)], do not produce similar tumor response profiles. There is no evidence of carcinogenicity at the low dose (10 ppm). Thus, even a complex computer simulation model of metabolism and pharmacokinetics that quantifies formation of carcinogenic metabolites is unlikely to provide a sufficient basis for risk prediction. The isoprene data may be seen as providing justification for the development of more complex "biologically-based" risk assessment models that seek to explicitly represent the nonlinear and/or time-varying dynamics of the interactions between metabolites and cell populations. Challenging as such models will be to build and validate, they may be needed to obtain usefully accurate descriptions of dose-time-response relations.

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